M and up to about 1 M. Binding of mercury and cadmium ions is effective over the pH range from 5 to 9, desirably from 6 to 8.5, and more preferably from 7 to 8.

Various techniques can be used to immobilize the chelon proteins on the surfaces of a solid support, provided that metal ion binding is not impaired due to the technique used for protein immobilization. The protein can be coated on the surface of the beads and/or interior surfaces of porous beads can similarly be coated. Chelon protein can be mixed with liquid phase material prior to gelling and bead formation. In a specifically exemplified embodiment, either chelon or MerR protein is linked to Streptactin Sepharose.

With poisoning with mercury, the enterohepatic recycling of mercury slows its elimination from the body. Since colonization of humans with a recombinant strain of bacteria is likely to face regulatory hurdles, an alternative strategy is the use of microspheres to which the chelon is covalently bound. Mounting the chelon protein in a microsphere with pores sufficiently large to allow diffusion of a low molecular weight thiols but sufficiently small to block diffusion of digestive proteases produces a robust high affinity and high specificity nonabsorbable sequestering agent. These microspheres pass readily through the intestinal tract, collecting mercury on the way to elimination via natural processes. An advantage of the chelon-containing microspheres of the present invention over prior art sulfonated amberlite resins is the increased specificity for the toxic metal over that of other divalent cations, such as copper or zinc, whose depletion would be deleterious to the human or animal host.

It is well known in the biological arts that certain amino acid substitutions can be made in protein sequences without affecting the function of the protein. Generally, conservative amino acid substitutions or substitutions of similar amino acids are tolerated without affecting protein function. Similar amino acids can be those that are similar in size and/or charge properties, for example, aspartate and glutamate and isoleucine and valine are both pairs of similar amino acids. Similarity between amino acid pairs has been assessed in the art in a number of ways. For example, Dayhoff et al. (1978) in *Atlas of Protein Sequence and Structure*, Volume 5, Supplement 3, Chapter 22, pages 345-352, which is incorporated by reference herein, provides frequency tables for amino acid substitutions which can be employed

as a measure of amino acid similarity. Dayhoff et al.'s frequency tables are based on comparisons of amino acid sequences for proteins having the same function from a variety of evolutionarily different sources.

The complete nucleotide sequence encoding the MerR protein of Tn21 is available on GenBank, Accession No. P07044. Plasmid pASK-IBA3 is commercially available from Sigma-Genosys (The Woodlands, TX). The StrepTagII (trademark of Institut fur Bioanalytik GmbH) technology (which depends on streptavidin binding by particular residues, i.e., WSHPQFEK amino acid residues present at the C-terminus of the recombinant chelon protein; amino acids 110-117 of SEQ ID NO:4) is described in United States Patent No. 5,506,121, incorporated by reference herein. The recombinant mercury-binding chelon expression plasmid is constructed by joining PCR amplicands of two copies of sequences encoding the metal binding domain (residues 81-127) of MerR. To facilitate joining of these 2 metal binding domains coding sequences in a direct tandem repeat, the primers used in the amplification are designed to have a common BamHI restriction endonuclease recognition site to allow the to be joined and also to include a linker regions between them (See Fig. 4). The outer primers add a BsaI site to each end, allowing the entre chelon coding sequence to be cloned into the vector pASK-IBA3 (at the BsaI site remaining after the short BsaI fragment is removed and both BsaI fragments were eliminated). The expression of the tagged protein is under the control of the tetracycline-inducible *tetA* promoter, and the polylinker into which a coding sequence of interest is cloned contains restriction endonuclease recognition sites for BsaI and BsmFI. Crude extracts of the recombinant *E. coli* cells containing the tagged chelon protein are treated to purify the tagged protein in accordance with instructions from the manufacturer.

Alternatively, controlled expression of the chelon can be achieved by inserting the coding sequence into pBAD (Invitrogen, Carlsbad, CA) for expression induced by arabinose.

The nucleotide sequence encoding the chelon protein in given in Table 1B. It is inserted into the vector for tetracycline-regulated synthesis at a BsaI restriction site.

For mercury ion-regulated expression of the chelon coding sequence, the chelon coding sequence is cloned using pCC306 [Condee and Summers (1992) *J. Bacteriol.* **174**:8094-8101]. The luciferase coding sequences are replaced with the chelon coding sequence. Chelon expression is regulated by mercury in conjunction with the naturally occurring MerR protein. There is no need for a tag to assist in purification because recombinant host cells with the mercury-regulated chelon expression plasmid are to be used without protein purification, at least in certain applications. This vector is based on the p15A replicon, and it is suitable for use in all Enterobacteriaceae. Such recombinant enteric bacteria (which are nontoxigenic and nonpathogenic) are suitable for use in the in vivo sequestration and elimination of mercuric ion from the human or animal gastrointestinal tract.

Techniques and agents for introducing and selecting for the presence of heterologous DNA, i.e., a chelon coding sequences operably linked to transcription and translation regulatory sequences functional in plant cells. in plant cells and/or tissue are well-known. Constitutive transcription regulatory sequences include the Cauliflower Mosaic Virus 35S and 19S promoters, and sequences which provide for efficient translational expression are described in, e.g., United States Patent No. 5,668,294 (Meagher et al.) And United States Patent No. 5,874,242 (Mensa-Wilmot). Where expression is preferred in above the ground plant parts, a rubisco promoter, for example, from soybean, can be used to drive chelon expression. The soybean rubisco promoter sequence is available from GenBank as Accession No. X58684. Genetic markers allowing for the selection of heterologous DNA in plant cells are readily available, e.g., genes carrying resistance to an antibiotic such as kanamycin, hygromycin, gentamycin, or bleomycin. The marker allows for selection of successfully transformed plant cells growing in the medium containing the appropriate antibiotic because they will carry the corresponding resistance gene. In most cases the heterologous DNA which is inserted into plant cells contains a gene which encodes a selectable marker such as an antibiotic resistance marker, but this is not mandatory. An exemplary drug resistance marker is the gene whose expression results in kanamycin resistance, i.e., the chimeric gene containing nopaline synthetase promoter, Tn5 neomycin phosphotransferase II and nopaline synthetase 3' non-translated region described by Rogers et al., *Methods for Plant Molecular Biology*, A. Weissbach and H. Weissbach, eds., Academic Press, Inc., San Diego, CA (1988).